

CLAIMS

What is claimed is:

✓ 1 A method for analyzing a nucleic acid sample comprising multiple loci, a
5 first locus having at least two possible allelic sequences and a second locus having at least three possible allelic sequences, said method comprising:

(a) combining at least a first and a second pair of oligonucleotide probes with said nucleic acid sample, said first pair being capable of hybridizing in proximity to each other within a segment of said nucleic acid sample comprising said first locus
10 and said second pair being capable of hybridizing in proximity to said second locus, wherein (i) the first member of each pair comprises a FRET donor and the second member comprises a FRET acceptor, wherein the FRET acceptor of the second member in said first pair has an emission spectrum which is different from the emission spectrum of the FRET acceptor of the second member of said second, (ii)
15 upon hybridization, the proximity of said first and second member of said probe pairs is sufficient to allow fluorescence resonance energy transfer between said FRET donor and said FRET acceptor, (iii) at least one of said members of said first pair has a sequence which results in the differential hybridization of that member with at least two different alleles which may be present at said first locus, and (iv) at
20 least one of said members of said second pair has a sequence which results in the differential hybridization of that member with at least three different alleles which may be present at said second locus;

(b) measuring the emission of each of said FRET acceptors at a first temperature; and

25 (c) repeating said emission measurements at a second and third temperature; wherein the emission of said FRET acceptors at different temperatures provides an indication of the alleles present at said first and second loci.

2 The method of Claim 1, further comprising a third pair of oligonucleotide
30 probes which is combined with said nucleic acid, wherein the FRET acceptor of each

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of the second members of each of the first, second and third probe pairs has an emission spectrum which is different from the emission spectrum of the others.

3. The method of Claim 1 or 2 wherein said nucleic acid sample is the product of one or more reactions selected from the group consisting of PCR, 3SR, SDA and RCA.

4. The method of Claim 1 or 2, wherein at least one probe pair member comprises two FRET acceptors, two FRET donors or a FRET acceptor and a FRET donor, and is a member of two different probe pairs.

5. The method of Claim 1 or 2, wherein steps (b) and (c) are repeated throughout a range of temperatures.

6. The method of Claim 5, wherein said range is from at least 20° C to at most 95° C.

7. The method of Claim 5, wherein steps (b) and (c) are repeated at least every 0.1 to 10 seconds.

8. The method of Claim 7, wherein the temperature is varied at least 0.01 to 1 °C per second.

9. The method of Claim 1 or 2, wherein said emission measurements at a particular temperature are simultaneous.

10. The method of Claim 1 or 2, wherein at least one of said FRET acceptors is selected from the group consisting of LC Red 640, Cy 5, Cy 5.5 and LC Red 705.

11. The method of Claim 1 or 2, wherein said emission measurements are corrected for spectral overlap between or among the different fluorophores.

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12. A method for analyzing a nucleic acid sample comprising three or more loci each having at least two different allelic sequences, said method comprising:

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10 (a) combining at least a first, a second and a third pair of oligonucleotide probes with said nucleic acid, each of the members of said pairs being capable of hybridizing in proximity to each other within a segment of said nucleic acid comprising at least one of said multiple loci, wherein (i) the first member of each pair comprises a FRET donor and the second member comprises a FRET acceptor, wherein the FRET acceptor of the second member in said first pair has an emission spectrum which is different from the emission spectrum of the FRET acceptor of said second and third oligonucleotide probe pairs, (ii) when said second and third probe pairs have the same FRET acceptor, each of said second and third probe pairs has a different T_m from each other for each different allele within the nucleic acid segment to which each member hybridizes (iii) upon hybridization, the proximity of the members of a probe pair is sufficient to allow fluorescence resonance energy transfer between said FRET donor and said FRET acceptor, and (iv) at least one of said members of each pair has a sequence which results in the differential hybridization of that member with at least two different alleles which may be present at said loci;

20 (b) measuring the emission of each of said FRET acceptors at a first temperature; and

(c) repeating said emission measurements at a second temperature; wherein the emission of said FRET acceptors at different temperatures provides an indication of the alleles present at said multiple loci.

25 ²/~~12~~. The method of Claim ¹/~~12~~, wherein the FRET acceptor of each of the second members of each of a first, a second and a third probe pair has an emission spectrum which is different from the emission spectrum of the others.

30 ³/~~14~~. The method of Claim ¹/~~12~~ or ²/~~13~~, wherein said nucleic acid sample is the product of one or more reactions selected from the group consisting of PCR, 3SR, SDA and RCA.

be present at each of said loci within said first amplification product and said second amplification product contains one of at least three or more different allelic sequences which may be present at each of said loci within said second amplification product, and wherein each of said amplification products comprises at least one member of a pair of FRET acceptor or FRET donor,

(b) contacting each of said loci specific amplification products with FRET labeled oligonucleotide probes wherein (i) each of said FRET probes hybridizes with said amplification product at a segment encompassing a specific locus, wherein each of said probes has a sequence complementary to one of the allelic sequences which may be present at said specific locus within said amplification products and the hybridization product of each FRET probe with the other allelic sequences which may be present at said specific locus in said amplification products contains one or more mismatches, insertions or deletions which result in differential T_m of the FRET probe from each of the possible allelic sequences within that locus in said amplification products, (ii) each of said FRET probes contains a member of a FRET donor and acceptor pair which is other than the FRET donor or acceptor contained in the corresponding specific amplification product with the proviso that one of the FRET acceptors has an emission spectrum which is different from the emission spectrum of the other FRET acceptor, and (iii) said primer sequence and oligonucleotide probe sequences are chosen so that upon hybridization said FRET donor and acceptor for each pair is in close proximity so as to allow fluorescence resonance energy transfer between said FRET donor and said FRET acceptor;

(c) measuring the emission of each of said FRET acceptors at a different wavelength at a first temperature; and

(d) repeating said emission measurements at a second and a third temperature;

wherein the emission of said FRET acceptors at said different temperatures provides an indication of the alleles present at said multiple loci.

24. The method of Claim 23, wherein said nucleic acid sample comprises three or more loci and is contacted with at least three PCR primers under conditions

which allow formation of at least three linear amplification products, and each of the FRET acceptors of a first, a second and a third probe donor and acceptor pair has an emission spectrum which is different from the emission spectrum of the others.

5 25. The method of Claim 23 or 24, wherein steps (b) and (c) are repeated throughout a range of temperatures.

26. The method of Claim 25, wherein said range is from at least 20° C to at most 95° C.

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27. The method of Claim 25, wherein steps (b) and (c) are repeated at least every 0.1 to 10 seconds.

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28. The method of Claim 27, wherein the temperature is varied at least 0.01 to 1 °C per second.

29. The method of Claim 23 or 24, wherein said emission measurements at a particular temperature are simultaneous.

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30. The method of Claim 23 or 24, wherein at least one of said FRET acceptors is selected from the group consisting of LC Red 640, Cy 5, Cy 5.5 and LC Red 705.

31. The method of Claim 23 or 24 wherein said emission measurements are corrected for spectral overlap between or among the different fluorophores.

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32. A method for analyzing a nucleic acid sample comprising:

(a) contacting a nucleic acid sample comprising at least three loci with at least three primers, each of which are specific for one of said loci, under conditions which allow formation of at least three linear amplification products which are specific for each of said loci, wherein each of said amplification products contains one of at least two or more different allelic sequences which may be present at each

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of said loci, and wherein each of said amplification products comprises at least one member of a FRET acceptor and FRET donor pair,

(b) contacting each of said loci specific amplification products with FRET labeled oligonucleotide probes wherein (i) each of said FRET probes hybridizes with said amplification product at a segment encompassing a specific locus, and the hybridization product of each FRET probe with the other allelic sequences which may be present within that locus in said amplification products contains one or more mismatches, insertions or deletions which result in differential T_m of the probe from each of the possible allelic sequences within that locus in said amplification products, (ii) each of said FRET probes contains a member of a FRET donor and acceptor pair which is other than the FRET donor or acceptor contained in the corresponding specific amplification product with the proviso that one of the FRET acceptors has an emission spectrum which is different from the emission spectrum of the other FRET acceptor, (iii) when the FRET donor and acceptor combination of different probes is the same, the T_m of the probe pairs from each different allele within the nucleic acid segment to which each probe hybridizes is different and (iv) said PCR primer sequence and oligonucleotide probe sequences are chosen so that upon hybridization said FRET donor and acceptor for each pair is in close proximity so as to allow fluorescence resonance energy transfer between said FRET donor and said FRET acceptor;

(c) measuring the emission of each of said FRET acceptors at a first temperature; and

(d) repeating said emission measurements at a second and third temperature; wherein the emission of said FRET acceptors at said different temperatures provides an indication of the alleles present at said multiple loci.

33. The method of Claim 32, wherein each FRET acceptor of the FRET donor and acceptor combination for a first, a second and a third probe has an emission spectrum which is different from the others.

34. The method of Claim 32 or 33, wherein steps (b) and (c) are repeated throughout a range of temperatures.

35. The method of Claim 34, wherein said range is from at least 20° C to at most 95° C.

36. The method of Claim 34, wherein steps (b) and (c) are repeated at least every 0.1 to 10 seconds.

37. The method of Claim 36, wherein the temperature is varied at least 0.01 to 1 °C per second.

38. The method of Claim 32 or 33, wherein said emission measurements at a particular temperature are simultaneous.

39. The method of Claim 32 or 33, wherein at least one of said FRET acceptors is selected from the group consisting of LC Red 640, Cy 5, Cy 5.5 and LC Red 705.

40. The method of Claim 32 or 33, wherein said emission measurements are corrected for spectral overlap between or among the different fluorophores.

41. A device for multichannel color analysis of a target nucleic acid amplification reaction comprising;

a chamber for holding a nucleic acid amplification reaction product comprising an optically transparent wall;

a source for providing electromagnetic radiation to said optically transparent wall;

at least four bandpass filters at least two of which are not coplanar, wherein said filters are positioned to simultaneously or sequentially filter fluorescence emissions from said chamber so as to provide filtered multichannel fluorescence signals; and

an optical detector positioned to receive said filtered emission signals.

42. The device of Claim 41 wherein said at least two bandpass filters are orthogonal to each other.

43. The device of Claim 41 wherein said chamber comprises a PCR, 3SR, SDA or RCA reaction chamber.

44. A device for multichannel color analysis of a nucleic acid amplification reaction comprising;

a chamber for holding a nucleic acid amplification reaction product comprising an optically transparent wall;

a source for providing electromagnetic radiation to said optically transparent wall;

at least three dichroic filters and two bandpass filters, wherein said bandpass filters are not coplanar and wherein said dichroic filters are positioned so that the emissions passing through each bandpass filter intersect each others path to simultaneously or sequentially filter florescence emissions from said reaction chamber so as to provide filtered multichannel florescence signals; and

an optical detector positioned to receive said filtered emission signals.

45. The device of Claim 44 wherein said chamber comprises a PCR, 3SR, SDA or RCA reaction chamber.